Improved Bleachability of Bagasses and Cotton Stalk Pulp by Xylanase Enzyme

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ABSTRACT

Among ten fungal strains; Aspergillus, Oryzae was the most effective strain for the production of Xylanase. The proper incubation period for the maximal production of xylanase, by Aspergillus and Oryzae were recorded after 5 days at 45°C. The activity of xylanase being 2.45 µ/m and biomass production were reached 5.88g/L under the same condition. Alternatively, Xylanase treatment improves accessibility of bleaching chemical to the pulps, decreasing diffusion resistance of the degraded lignin As a result, pulps treated with xylanase show lower kappa number and higher brightness than the pulps, not treated with the enzyme Also, strength properties of paper increased clearly after treatment by enzyme)breaking length raised from 1511.6 to 2325.1m for bagasse and from 1000.5 to 1690.9m for cotton stalk .Infra red spectra & electron microscope were also studied

Introduction

During the last 30 years the paper industry had intensitied its efforts to minimize pollution arising from pulping and bleaching processes. Microorganisms perform their myriads of biochemical reactions under ambient conditions with little or no toxic and hazardous by products. Therefore enzyme alternatives to polluting chemical technologies can be considered as practical technologies of the future[1]. One of the most important large-scale biotechnological applications of recent years is the use of xylanases as bleaching agents in the pulp and paper industry[2]. Many researchers reported that xylanases decrease the use of chlorine needed for bleaching kraft pulp and play an important role when the use of hazardous chemical is to be decreased in bleaching processes[3-4]. Pulping and bleaching are both performed at high temperature. Hence the paper industry needs xylanases that are thermostable and preferably, active at neutral and alkaline pH[5]. Also xylanases are mostly contaminated with cellulases, which destroy the structure of cellulose and diminish pulp quality. This means xylanases with a high degree of cellulase-free xylanase for large scale pulp bleaching biotechnology require effects that are aimed at process optimization, simplification and cost reduction. The production of xylanases must be improved by finding potent fungal or bacterial strains or by inducing mutant strains to produce and excrete greater amount of enzymes or by enhancing production by solid state fermentation (SSF) [6]. The bleaching efficiency of xylanases is measured either as the reduction in the amount of chemicals used for bleaching of pulp or brightness gain induced by enzyme[2]. In the paper production process, pulping is a step during which cellulose fibers are broken apart and most of lignin is removed. Our earlier study on chemical pulping for pulp and paper making, has shown that the alkaline process using sodium hydroxide alone appears to be the most interesting when its pulping efficancy and its environmental friendliness (in comparison with either sulphite or kraft process)are taken into account[7,8].

In this work hand sheet paper were prepared from bagasse and cotton stalk after pulping by 12% sodium hydroxide, then treated by enzymes. Strength and optical properties, Infra- red as well as scanning electron microscopy were studied for the prepared paper.

Metril & Methods

Raw materials

Bagasse and cotton stalk were the raw material used in this work.

Pulping

Pulping was done by using soda process (12% sodium hydroxide),in an heated autoclave (two revolutions per min.). Time had taken for pulping 1 h, 1:6 liquor ratios (dry raw material: solution respectively) and the pulping temperature 140°C

Fungal Strains Used

Ten fungal strains were obtained from culture collection of Microbial Chem. Dep., Nat. Res. Center, Dokki, Giza, Egypt. They were belonged to five genera and different species, namely *Aspergillus* (spp. : *oryzae* DSM 186 and 185, *niger* 66/200, *flavus* B/lOS, *ochraceus 67/33), Fusarium (moniliforme 24), Penicillium (funiculosum NRRL 6014), Rhizopus (oligosporus 82) and Trichoderma (reesei 15 and viride 36).*

Media Used

peptone yeast agar medium [9] used for maintenance, propagation and growth of the tested fungal cultures (Czapek-Dox medium [10] used for fungal production of The cellulases

and xylanase: Ten g/L of cellulose (Carboxymethyl Cellulose) or filter- paper [Whatman No. 1] or xylan was added to the medium for the production of xylanase enzyme.

Enzyme Activity
1- Qualitative Methods

Xylanase was determined according to Morkbak et al. [11].

2- Quantitative Methods
D- Xylanase Activity:

Xylanase estimation was done according to method adopted by Garg et al.[14]. The reducing sugars were estimated using dinitrosalicylic acid (DNS) method [11], using D-xylose as standard. One unit of xylanase activity is defined as the amount of enzyme that is capable of liberating 1 mole of xylose in 1min.

Fermentations

Fermentations were carried out in 250ml conical flasks. Each contained 40 ml of basal medium The experimental medium was inoculated as mentioned above. The experimental culture flasks were incubated at 30°C using rotary shaker at 150 rpm. The content of each experimental flask was taken daily and centrifuged at 5000 rpm for 20 min using a cooling centrifuge (Janetzki, k-26). The filtrates were used for analysis of the extra cellular enzymes. Bleaching of pulp by fungal enzymatic treatments, it was carried out according to the method recommend by Zhang et al. [15].

Table (1): Effect of different incubation periods on the production of xylanase by some fungal strains

Fungal strains	Enzymes	Incubation period (day)							
		1	2	3	4	5	6	7	8
Aspergilus oryzae DSM 186	Xylanase	-	-	-	+	+++	+++	+++	+++
A. oryzae 185	Xylanase	+	+	+	+	++	++	++	++
A.niger 66/200	Xylanase	-	+	+	+	++	++	++	++
A. flavus B/105	Xylanase	-	-	-	-	+	+	+	+
A. ochraceous 67/33	Xylanase	-	-	-	-	-	-	-	-
Fusarium moniliforme 24									
Penicillium funiculosum	Xylanase	+	+	+	+	++	++	++	++
NRRL 6014	Cellulase	-	+	+	+	++	++	++	++
Rhizopus oligosporus 82	Xylanase	++	++	++	++	++	++	++	++
Trichoderma reesei 15	Xylanase	+	+	+	+	+	+	+	+
T. viride 36	Xylanase	+	+	+	+	+	+	+	+

Paper making

The biobleached pulps (control or enzymatic) were beaten in a jardan beater at 3.6 consistency until they reached 38-40 S°R. Sheet were prepared according to tappi standard method using the sheet former of AB Lorentzen and wetter (Stockholm, Sweden) After sheet forming, the papers were conditioned for 24h at 20°C and 50%RH,and then measured the optical properties[16] (Brightness and Opacity) and mechanical properties[16] (breaking length and brusting strength).

Kappa number

It was calculated according to scan-C[17]

Infrared (IR)

IR absorption of the paper samples were recorded using an FT/IR 300 Elascousing KBr discs.

Scanning Electron Microscope (SEM)

Apiece of paper covered with gold from one face and the other face put on the holder, then enter in the scanning electron microscope (JEOL JXA-840 A, Electron micro analyzer).

Result and Discussion
Delignification by Soda Pulping

Lignin consists mainly of phenyl propane unit linked together in three dimensions, during soda pulping process these three linkages between propane side chains and the benzene rings are broken which results in setting the cellulose fiber [19]. The swollen lignin is split into fragment by the hydroxyl ions(OH). The lignin fragments are dissolved as phenolate or carbohydrate, so the lignin is reduced.

Screening of some fungal strains for their bleaching ability and removing lignin

Studying of some nutritional and physical conditions influencing the production of enzymes (cellulase and xylanase) responsible for removal of lignine and bleaching of soda pulp bagasse and cotton stalk using ten fungal strains.
Data presented in table (1) show that, *Aspergillus Oryzae* DSM186 had produced the highest amount of both xylanase and cellulase enzymes after 5 days of incubation at 45^{0}C, the activity of xylanase and cellulase recorded after 5 days at 45^{0}C were 2.45 M/mL. the biomass production reached 5.88 and 4.2 g/L for xylanase and cellulase respectively under the same condition.

This result confirmed with that obtained by Baitey et al. [18]. Since they mentioned that *Aspergillus oryzae* could produce xylanase on both cellulose and xylan based media.

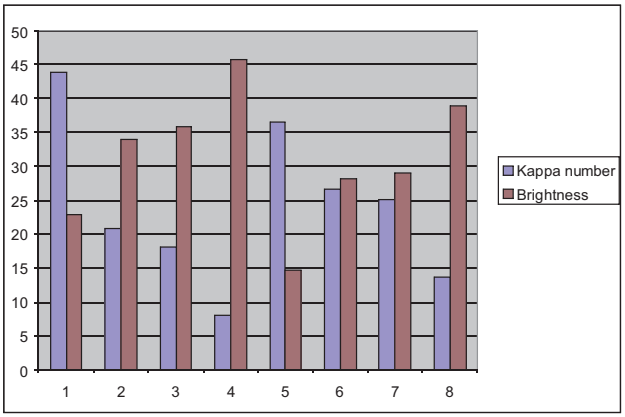
Levels of xylanase Productions (Lytic Zones in mrn): No "-" (0 mm), Weak "+" (>0-10 mm), Moderate "++" (1 1-20 mm) and High "+++" (21-30 mm).

Kappa number and brightness of different type of pulps

It is clear from Fig (1) that xylanases treated bagasses pulp was greatly enhanced kappa number, which reduced by 82% & 76% in case of treated bagasse pulp and treated cotton stalk pulp respectively. As a result from Fig (1) pulps treated with xylanase show greater brightness which increased by 23.1% and 36.9% in case of bagasse pulp and cotton stalk pulp treated enzyme respectively. This is due xylanasees may break down the lignin carbohydrate bonds, improving the extractability of solubilized lignin [20].

Alternatively, xylanase treatment improves accessibility of bleaching chemical to the pulps, decreasing diffusion resistance of the degraded lignin fragments allowing lignin removal from the cell wall. As a result, pulps treated with xylanase show lower kappa number and higher brightness than the pulps, not treated with the enzyme [21]. The evidence is in agreement with our previous work [22-23].This means that xylanase treatment improved bleachability & did not affect the quality of the pulp. Such results are better than those of previously reported enzymes by some workers [24, 25]. Treatment bagasse and cotton stalk by 12% soda, decreased kappa number by 52.7 and 52.5 respectively. Also treatment soda pulp of bagasse and cotton stalk by xylanase enzyme, improved the brightness by 34.7% and 35.7% respectively.

Fig 1: Brightness and Kappa number



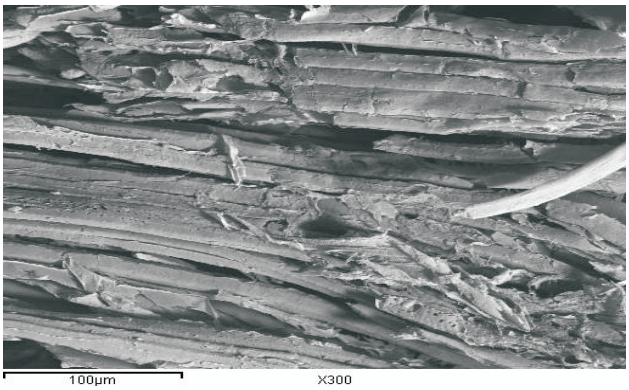
1-Bagasse (raw material) 2- Pulped Bagasse soda) (12%)
3-Control Bagasse pulp 4-Enzymatic Bagasse pulp
5-Cotton Stalk (raw material) 6-Pulped Cotton stalk (12% Soda)
7-Control Cotton Stalk pulp 8-Enzymatic Cotton Stalk pulp

Scanning electron microscope (SEM)

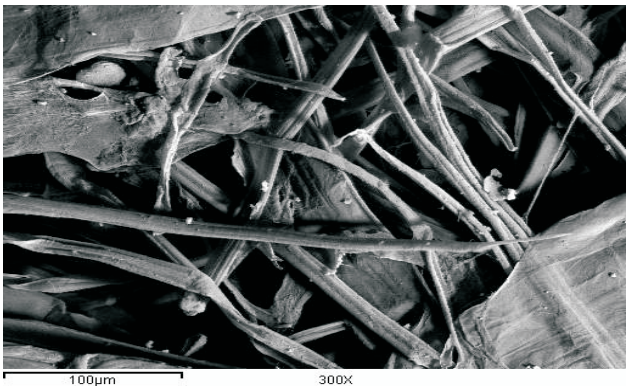
Fig. (2, 3) show that photographs of different pulps of bagasse and cotton stalk.

It was clear that photographs Fig. (2 a) and Fig. (3e) show many more filaments and fibrils these being more pronounced in soda plup (2b) & (3f). While photographs fig. 2d & Fig. 3h the pulp treated with enzyme show a cleaner pulp with no of fibrillation or filaments between the fibers, no cracks or voids, thus the crack does not have a straight path because it has to move around the fiber cells & ultimately stops [26]. While control pulp. Fig. (2c) & Fig. (3g) of bagasse and cotton stalk show more void and cracks than pulps treated by enzyme. Enzyme has a great effect on the surface of the fibre and significant changes were indicated by SEM as a result of xylan hydrolysis. All of fibrillar material was eliminated and an apparent high

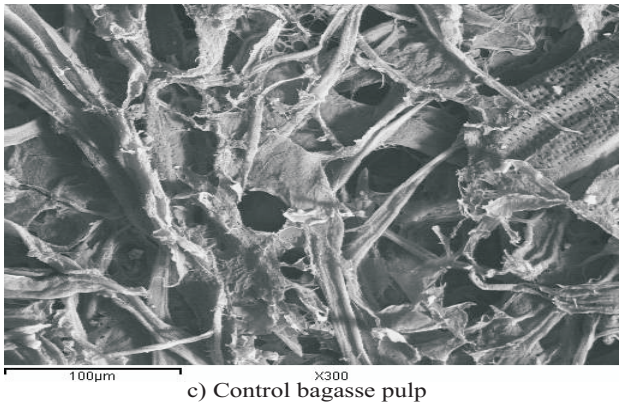
Fig (2): Scanning electromicroscope bagass



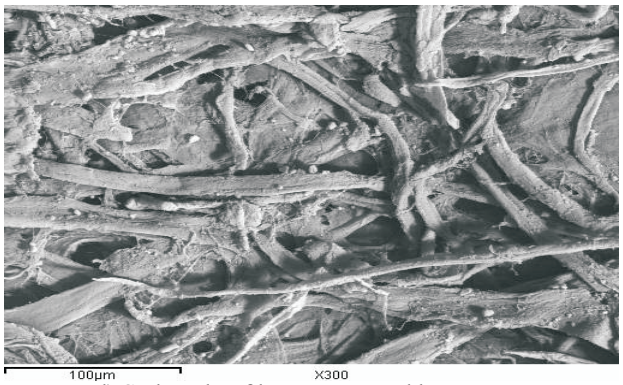
a) bagasse raw material



b) Soda pulp of bagasse

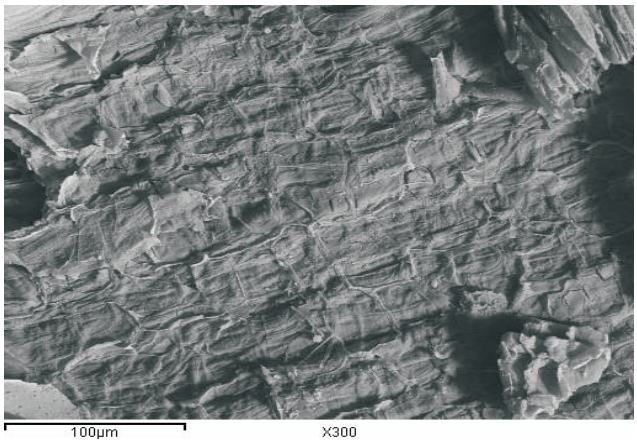


c) Control bagasse pulp

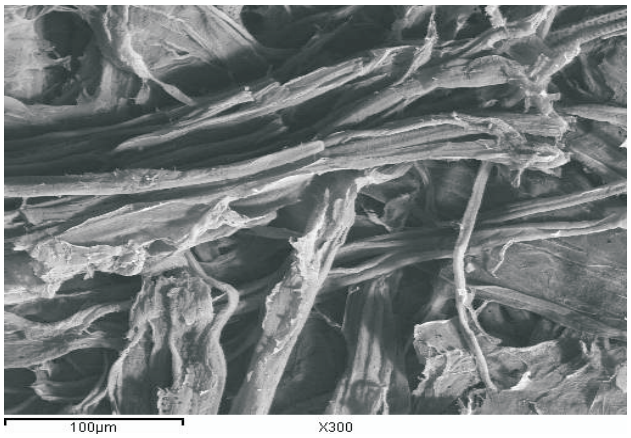


d) Soda pulp of bagasse treated by enzyme

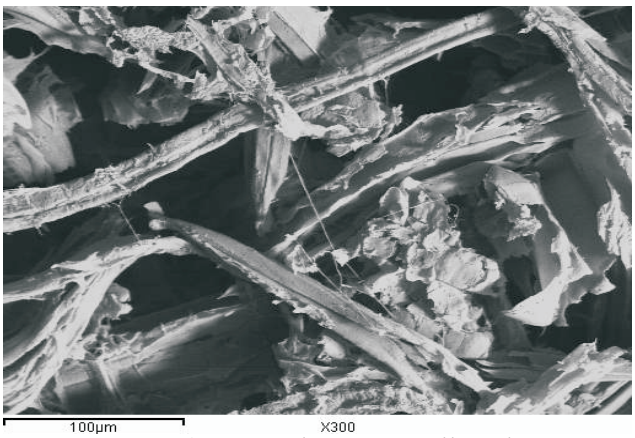
Fig (3): Scanning electron microscope of cotton stalk



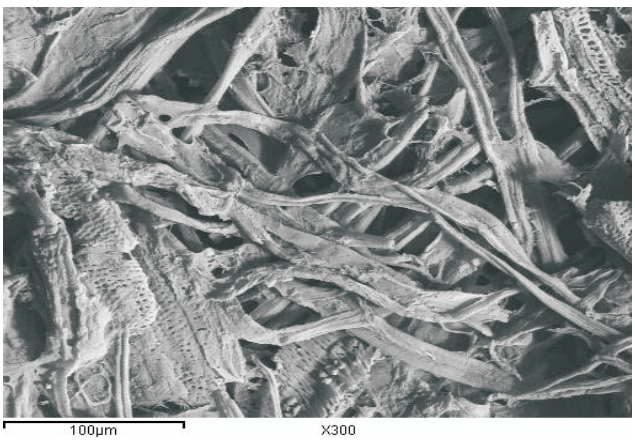
e) Cotton stalk



f) Soda pulp of Cotton stalk



g) Control cotton stalk pulp



h) Soda pulp of Cotton stalk treated by enzyme

Table (2): Characteristic bands of infrared spectra of bagasse and cotton stalk

Wave number cm^{-1}	Assignment
3450	OH stretching (H-bonded)
2885	Alkyl CH stretching
1740	C=O stretching of hemicellulose
1630	H-OH bond or C=C
1507	Aromatic bending C-H (ring)
1465	Lignin and CH_2 sym, bending pyran ring
1426	CH_2 bending (cell) (12.51 – 12.65Asymmetric C-O-C)
1331	OH in plan bending (cell)
1162	Antisym, bridge C-OR-C stretching (cell)
1110	C-O stretching (C-OH)
1055	Stretching C-OR (cell)
898	Antisym, out of phase ring stretching.

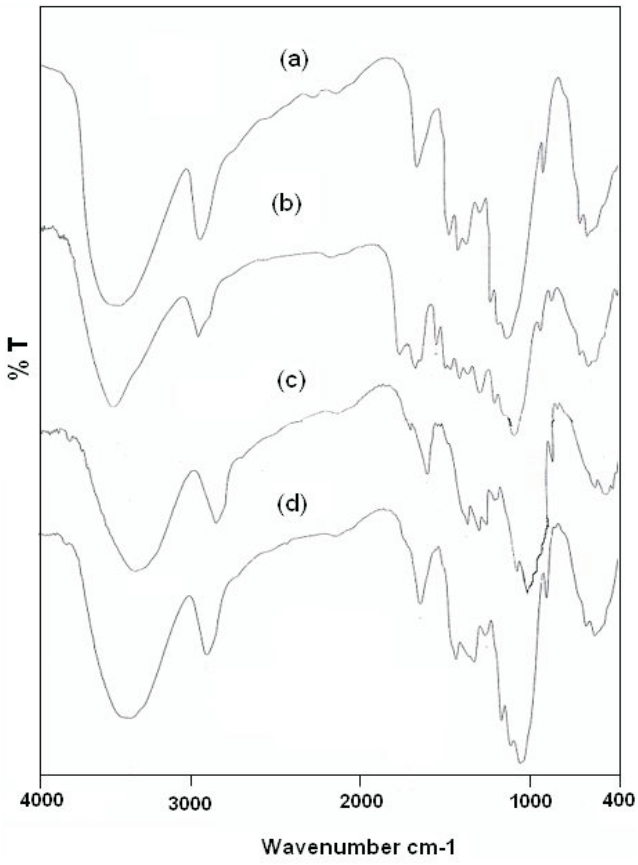
flexibility and conformability which contribute to good bonding were more visible. These results may be due to cell wall swelling and softening.[27]

Infra red spectra

FTIR spectra of bagasse and cotton stalk pulp are presented in figs. 4 & 5. The band positions and possible assignments are presented in table (2) From Figs. (4 & 5) it can be observed that well defined bands around 3400, 2900, 1740, 1650, 1250, 1110, and 1050 cm^{-1} are present in the spectra from Table (2), it is evident that the bands around 3400 and 2900 cm^{-1} correspond to OH stretching of -cellulase. The band around 1740 cm^{-1} corresponds to CO stretching of hemicellulose content.

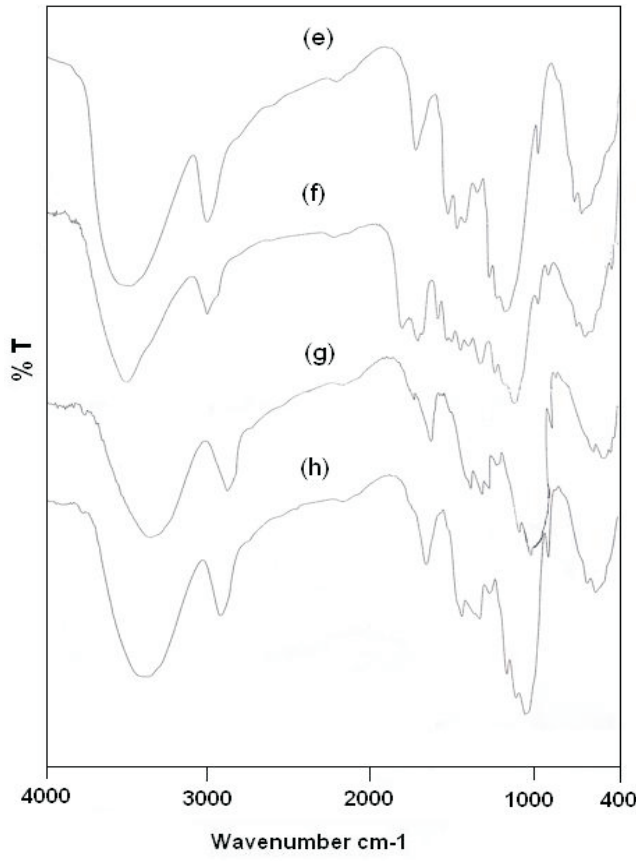
The remaining bands belong to CO and C-O-C stretching of lignin. It is clear that from Fig. 4a and 5e corresponds to 12% soda pulp of bagasse & cotton stalk, respectively after the removal of hemicellulose to a considerable extent. The percentage of α - cellulose is expected to increase. The increase in the intensity of the bands around 3400 and 2900 cm^{-1} for the 12% soda pulp of both bagasse and cotton stalk respectively. Fig. (4a) and Fig. (5e). We found also in fig 4c &5g that the

Figure (4) Infrared spectra of bagasse



a) Bagasse soda pulp b) Bagasse raw material
c) Bagsse pulp treated by enzyme d)Control bagasse pulp

Figure (5) Infra red spectra of Cotton stalk



e)Cotton stalk soda pulp f)Cotton stalk raw material
g) Cotton stalk pulp treaded by enzme h) Control Cotton stalk pulp

hydroxyl band decreased along with C-O-C band and suggests that xylanases broke and removed xylose-oligosaccharide portions from xylan of pulp, resulting in lower amounts of OH lips and C-O-C bond in the pulp.[28]

Hand-sheets from Enzymatic pulp

Table (3) shown that the xylanase enzyme improved the bleachability of hand-sheet made from soda pulp either bagasse or cotton stalk, compared to control pulp. The brightness increased by 9.9% for bagasse and cotton stalk. This due to the xylanase enzyme has ability to degrade lignin and removed from pulp[21]. Also, strength properties of paper increased clearly after treatment by enzyme (breaking length raised from 1511.6 to 2325.1m for bagasse and from 1000.5 to 1690.9m for cotton stalk compared to control pulp. Enzymes formed filament which enter with fiber and this lead to increase strength properties.[29]

Table (3): Optical and strength properties of the paper prepared from bagasse and cotton stalk.

Properties	Optical properties		Strength properties	
Sample	Brightness %	Opacity %	Breaking length m	Bursting strength kg/cm^2
Control bagasse pulp	35.9	92	1511.6	1.1
Enzymatic bagasse pulp	45.8	94.1	2325.1	2.05
Control cotton stalk pulp	29	90.4	1000.5	0.8
Enzymatic cotton stalk pulp	38.9	92	1690.9	1.26

Conclusions:

1. Treatment bagasse and cotton stalk by 12% soda decreased kappa number to a large extent.
2. Enzymatic treatment soda pulp of bagasse and cotton stalk, improved bleachability clearly and enhanced properly the strength properties of pulp.

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